AN EPR DATA SYSTEM BASED ON S-100 BUS HOME COMPUTER COMPONENTS

This article presents a technique for construction of a laboratory data system using commercially available S-100 (IEEE-696) bus components. The system was designed to work specifically with an IBM/Bruker ESR spectrometer, but the design is flexible enough that the system could easily be used with a variety of laboratory instruments. The software package which was written for this system allows the user to acquire either analog or digital data from a laboratory instrument, signal average the analog data and store the data on disk files. The S-100 bus components are inexpensive and one is able to construct a complete data system at minimum cost. A final part of this article describes software which has been developed for processing EPR data.

Schultz, R., Hurst, G., Thieret, T. E., and *Kreilick, R. W.*

Journal of Magnetic Resonance 53:303-312, 1983.

Other support: National Institutes of Health.

From the Department of Chemistry, University of Rochester, Rochester, NY.

DIMETHONIUM, A DIVALENT CATION THAT EXERTS ONLY A SCREENING EFFECT ON THE ELECTROSTATIC POTENTIAL ADJACENT TO NEGATIVELY CHARGED PHOSPHOLIPID BILAYER MEMBRANES

The summary of the paper presented here states that calcium and other alkaline earth cations change the electrostatic potential adjacent to negatively charged bilayer membranes both by accumulating in the aqueous diffuse double layer adjacent to the membrane and by adsorbing to the phospholipids. The effects of these cations on the electrostatic potential are described adequately by the Gouy-Chapman-Stern theory. The investigators report the results of experiments with ethane-bis-trimethylammonium, a cation that has been termed "dimethonium" or "ethamethonium" in analogy with hexamethonium (hexane-1,6-bis-trimethylammonium) and decamethonium (decane-1,10-bis-trimethylammonium). They examined the effect of dimethonium on the zeta potential of multilammellar vesicles formed from the negative lipid phosphatidylserine (PS) and from 5:1 phosphatidylcholine/phosphatidylserine mixtures in solutions containing 0.1, 0.01 and 0.001 M sodium, cesium, or tetramethylammonium chloride. The researchers also examined the effect of dimethonium on the conductance of planar PS bilayer membranes and the ^{31}P NMR signal from sonicated PS vesicles formed in 0.1 M NaCl. They found no evidence that dimethonium adsorbs specifically to bilayer membranes. All the results, except for those obtained with vesicles of low charge density formed in a solution with a high salt concentration, are consistent with the predictions of the Gouy-Chapman theory. The investigators conclude that dimethonium, which does not have the pharmacological effects of hexamethonium and decamethonium, is a useful divalent cation for physiologists interested in investigating electrostatic potentials adjacent to biological membranes.

McLaughlin, A. et al.

The Journal of Membrane Biology 76:183-193, 1983.

Other support: National Institutes of Health and the National Science Foundation.

From the Biology Department, Brookhaven National Laboratory, Upton, NY, and the Department of Physiology and Biophysics, Health Sciences Center, State University of New York, Stony Brook.

LARGE DIVALENT CATIONS AND ELECTROSTATIC POTENTIALS ADJACENT TO MEMBRANES: EXPERIMENTAL RESULTS WITH HEXAMETHONIUM

As presented here, a simple extension of the Gouy-Chapman theory predicts that the ability of a divalent cation to screen charges at a membrane-solution interface decreases significantly if the distance between the charges on the cation is comparable with the Debye length. The researchers tested this prediction by investigating the effect of hexamethonium on the electrostatic potential adjacent to negatively charged phospholipid bilayer membranes. The distance between the two charges of an extended hexamethonium molecule is ~1nm, which is the Debye length in the 0.1 M monovalent salt solutions used in these experiments. Six different experimental approaches were utilized. The investigators measured the electrophoretic mobility of multilamellar vesicles to determine the zeta potential, the line width of the ^{31}P nuclear magnetic resonance (NMR) signal from sonicated vesicles to calculate the change in potential at the phosphodiester moiety of the lipid, and the conductance of planar bilayer membranes exposed to either carriers (nonactin) or pore formers (gramicidin) to estimate the change in potential within the membrane. They also measured directly the effect of hexamethonium on the potential above a monolayer formed from negative lipids, and attempted to calculate the change in the surface potential of a bilayer membrane from capacitance measurements. With the exception of the capacitance calculations, each of the techniques gave comparable results: hexamethonium exerts a smaller effect on the potential than that predicted by the classic screening theory. The results are consistent with the predictions of the extended Gouy-Chapman theory and are relevant to the interpretation of physiological and pharmacological experiments that utilize hexamethonium and other large divalent cations.

Alvarez, O., Brodwick, M., Latorre, R., *McLaughlin, A.*, McLaughlin, S., and Szabo, G.

Biophysical Journal 44:333-342, 1983.

Other support: University of Chile, National Institutes of Health and the National Science Foundation.

From the Departamento de Biologica, Facultad de Ciencias Básicas Farmacéuticas, Universidad de Chile, Santiago; Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston; Department of Physiology and Biophysics, Harvard Medical School, Boston; Biology Department, Brookhaven National Laboratory, Upton, NY; and Department of Physiology and Biophysics, Health Sciences Center, State University of New York, Stony Brook.

INCREASED SISTER-CHROMATID EXCHANGE IN BONE MARROW CELLS OF MICE EXPOSED TO WHOLE CIGARETTE SMOKE

Previous studies have shown that exposure of BC3F1/Cum female mice to whole cigarette smoke resulted in the induction of the pulmonary macrosomal enzymes, aryl hydrocarbon hydroxylase, ethoxyresorufin-*O*-deethylase, and ornithine decarboxylase. In the study presented here, using defined cigarette smoke exposure conditions, BC3F1/Cum mice were exposed nose-only to two different types of whole cigarette

smoke on a daily basis for or
chromatid exchanges (SCEs) p
Studies were scheduled so that
the last smoke exposure. Expos
or up to 46 weeks resulted in
mice. In animals exposed eithe
the increase in SCEs persisted
This is the first demonstration
been exposed to cigarette smo

Benedict, W. F. *et al.* (*Micro*

Mutation Research 136:73-80

From the Division of Hemat
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Mitchell, J. A. and Hamn

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From the Department of
Detroit.

smoke on a daily basis for one week and up to 46 weeks. The number of sister-chromatid exchanges (SCEs) per metaphase was determined in bone-marrow cells. Studies were scheduled so that all cytogenetic observations were made 2-3 days after the last smoke exposure. Exposure to either type of smoke on a daily basis for one week or up to 46 weeks resulted in a 2-fold increase in SCEs over sham-exposed control mice. In animals exposed either chronically or for one week to either type of smoke, the increase in SCEs persisted for at least one week after cessation of smoke exposure. This is the first demonstration of the induction of SCEs in laboratory animals that have been exposed to cigarette smoke *in vivo*.

Benedict, W. F. *et al.* (*Microbiological Associates*)

Mutation Research 136:73-80, 1984.

From the Division of Hematology-Oncology, Department of Medicine, Children's Hospital of Los Angeles, and Department of Pediatrics, University of Southern California School of Medicine, Los Angeles; and Division of Toxicology and Oncology, Microbiological Associates, Bethesda, MD.

EIN VERGLEICH DER WIRKUNGEN VON NIKOTIN UND SEROTONIN AUF PERIIMPLANTATIONSPHÄNOMENE IN DER RATTE

In previous investigations we established that the vasoactive substances nicotine and serotonin clearly have distinguishable actions on the course of early pregnancy in rats: nicotine reduced the growth of the blastocyst, delayed the loss of the zona pellucida and retarded implantation without impairing fertility. In contrast, serotonin destroyed the integrity of the implantation site and was embryotoxic. Since prior to implantation the fertilized egg is free living in the lumen of the reproductive tract and is, therefore, fully dependent on diffusion for its nourishment and the exchange of metabolic products, the blastocyst is highly susceptible to changes in the intraluminal environment. The following experiments were carried out to compare the actions of nicotine and serotonin on uterine blood flow and on intrauterine oxygen tension. The actions of a single subcutaneous injection of 5 mg/kg nicotine, 20 mg/kg serotonin or saline (as control) on blood flow at the implantation site of pregnant rats on Day 5 of pregnancy and on oxygen tension on Day 4 of pseudopregnancy were determined. Blood flow was measured with ^{86}Rb (rubidium); intraluminal pO_2 was determined polarimetrically. Results indicate that while the actions of both nicotine and serotonin on implantation site blood flow and intrauterine pO_2 were comparable both in degree and duration, their actions on blastocyst survival were clearly different. It is concluded that blastocysts can survive protracted oxygen deficiency following serotonin or nicotine-induced vasoconstriction. Thus, the embryotoxic effects of serotonin result from some action(s) of the amine other than reduced implantation site blood flow and concomitant intrauterine hypoxia.

Mitchell, J. A. and Hammer, R. E.

Verhandlungen der anatomischen Gesellschaft 77:425-426, 1983.

From the Department of Anatomy, Wayne State University School of Medicine, Detroit.

QUANTIFICATION OF GUANYLATE CYCLASE CONCENTRATIONS BY A DIRECT DOUBLE DETERMINANT TANDEM IMMUNORADIOMETRIC ASSAY

In this paper, the authors have described the development and application of a simple and direct tandem immunoradiometric assay for quantitating guanylate cyclase protein, independent of enzyme activity, in crude samples. Since the assay uses two antiguanylate cyclase monoclonal antibodies directed to different determinants on the protein, it is a very specific assay. To be exact, a total of 16 monoclonal antibodies have been produced to soluble guanylate cyclase. Two of the antibodies, designated H_6 (IgG_{2a} subclass) and B_4 (IgG_1 subclass), were iodinated and the characteristics of their binding to immobilized guanylate cyclase were examined. Scatchard transformations of binding data were noted. Competitive binding studies revealed that antibodies H_6 and B_4 recognize different determinants on the enzyme. The two antibodies were used to develop a direct, double determinant tandem immunoradiometric assay for soluble guanylate cyclase based on the differential binding of mouse immunoglobulin subclasses to Protein A on *S. aureus* membranes. The investigators also used this assay to quantitate soluble guanylate cyclase protein, independent of enzymatic activity, in a variety of rat tissues. These studies showed lung to be a rich source of the enzyme. Measurements of guanylate cyclase protein were not altered by agents that activate (sodium nitroprusside) or inactivate (cystamine) the enzyme. The ability to measure guanylate cyclase protein, independently of catalytic activity or cyclic GMP levels, should prove extremely useful in studying the function and regulation of this enzyme-nucleotide system.

Lewicki, J. A., Chang, B. and *Murad, F.*

The Journal of Biological Chemistry 258(6):3509-3515, 1983.

Other support: National Institutes of Health.

From the Departments of Medicine and Pharmacology, Stanford University, and Palo Alto Veterans Administration Medical Center, Palo Alto, CA.

PARTIAL PURIFICATION AND CHARACTERIZATION OF PARTICULATE GUANYLATE CYCLASE FROM RAT LIVER AFTER SOLUBILIZATION WITH TRYPSIN

As summarized here, guanylate cyclase from 105,000 × g particulate fractions of rat liver homogenates (20 pmoles of cyclic GMP formed/min/mg protein) was solubilized in the absence of detergents by incubating fractions 12 min at 37° with 5 ug/ml trypsin. Optimal solubilization was dependent upon trypsin and particulate preparation concentrations. Virtually no activation of particulate guanylate cyclase was observed at any time point or trypsin concentration tested. Guanylate cyclase solubilized with trypsin was purified about 500-fold (9.4 nmoles/min/mg protein) using ammonium sulfate precipitation, GTP-affinity chromatography, and preparative polyacrylamide gel electrophoresis. Activity eluted as a single peak on Sephadex G-200 (Stokes radius = 40Å) and migrated as a single peak on sucrose density gradients ($S_{20,w}$ = 4.6). Thus, the tryptic fragment was estimated to be about 80,000 daltons (Mr) with a frictional ratio (f/f_0) of 1.4. These partially purified preparations exhibited linear double reciprocal plots with Mn-GTP and Hill coefficients of 1.0. This is in contrast to the crude membrane-associated enzyme which had a Hill coefficient of 1.5. These and other

studies indicate that particulate gua
amenable to purification by "clas
purified fragment contains the cata
least one sulfhydryl group required

Waldman, S. A., Lewicki, J. A.,

Journal of Cyclic Nucleotide Rese

Other support: National Institutes

From the Departments of Medicin
cology, Stanford University Veter

EFFECT OF OUABAIN AND A
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of sodium nitroprusside on relaxa
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may induce relaxation through c
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Rapoport, R. M. and *Murad, F.*

Blood Vessels 20:255-264, 1983

Other support: National Institut

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studies indicate that particulate guanylate cyclase solubilized by limited proteolysis is amenable to purification by "classical" chromatographic techniques. The partially purified fragment contains the catalytic site, the site for nitric oxide activation, and at least one sulfhydryl group required for activity.

Waldman, S. A., Lewicki, J. A., Brandwein, H. J., and *Murad, F.*

Journal of Cyclic Nucleotide Research 8(6):359-370, 1982.

Other support: National Institutes of Health.

From the Departments of Medicine and Pharmacology, Division of Clinical Pharmacology, Stanford University Veterans Administration Hospital, Palo Alto, CA.

EFFECT OF OUABAIN AND ALTERATIONS IN POTASSIUM CONCENTRATION ON RELAXATION INDUCED BY SODIUM NITROPRUSSIDE

The purpose of the present study was to investigate (1) whether relaxation induced by nitroprusside is effected by agents and procedures known to alter the activity of the Na^+, K^+-pump, and (2) if relaxation to nitroprusside was effected, whether the changes induced by nitroprusside could be mediated through the formation of cyclic GMP. Results of this study show that relaxation of the rat thoracic aorta induced by sodium nitroprusside, 8-bromo-cyclic GMP and cyclic nucleotide phosphodiesterase inhibitor M & B 22,948 was inhibited by exposure to K^+-free solution and ouabain in a concentration-dependent manner. Relaxation occuring with the change in potassium concentration in media from 1 to 2 to 10 mM was increased by sodium nitroprusside, 8-bromo-cyclic GMP and M & B 22,948. Thus, agents and procedures known to decrease and increase the activity of the Na^+,K^+-pump, and presumably alter the membrane potential, inhibited and enhanced relaxation, respectively. Exposure to 1 mM K^+ solution increased the relaxation to low concentrations of sodium nitroprusside, but had no effect on relaxation induced by 8-bromo-cyclic GMP or M & B 22,948. Thus, another procedure which inhibits the Na^+,K^+-pump enhanced the effect of low concentrations of sodium nitroprusside on relaxation. Ouabain had no effect on sodium nitroprusside-induced accumulation of cyclic GMP. These results suggest that sodium nitroprusside may induce relaxation through cyclic GMP formation, effects on the Na^+, K^+-pump and/or hyperpolarization of the smooth muscle cell membrane.

Rapoport, R. M. and *Murad, F.*

Blood Vessels 20:255-264, 1983.

Other support: National Institutes of Health.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine and Veterans Administration Medical Center, Palo Alto, CA.

AGONIST-INDUCED ENDOTHELIUM-DEPENDENT RELAXATION IN RAT THORACIC AORTA MAY BE MEDIATED THROUGH cGMP

The purpose of this study was to test the hypothesis that relaxation of vascular smooth muscle with other agents that are dependent upon the endothelium, may also be

mediated through the formation of cGMP within the smooth muscle. Results showed that relaxation of the rat thoracic aorta to acetylcholine, histamine, and Ca^{++} ionophore A23187 was associated with increased levels of cGMP in a time- and concentration-dependent manner, whereas cAMP levels were unaltered. Removal of the endothelium prevented relaxation to these agents and prevented the increased levels of cGMP. Removal of the endothelium after exposure to acetylcholine only partially decreased the elevated levels of cGMP, suggesting that the changes in cGMP occurred within the smooth muscle cells. Eicosatetraenoic acid, an inhibitor of lipoxygenase and cyclooxygenase, and quinacrine, an inhibitor of phospholipase, prevented and reversed acetylcholine-induced relaxation, respectively, and inhibited acetylcholine-induced increased levels of cGMP. In contrast, sodium nitroprusside-induced relaxation and increased levels of cGMP were independent of the presence of the endothelium, exposure to eicosatetranoic acid and quinacrine. The present results support the hypothesis that vascular smooth muscle relaxation induced by some agents is dependent on the presence of the endothelium and is mediated through the formation of an endothelial factor that increases cGMP levels in smooth muscle.

Rapoport, R. M. and *Murad, F.*

Circulation Research 52:352-357, 1983.

Other support: National Institutes of Health, Veterans Administration and a National Research Service Award.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Palo Alto Veterans Administration Medical Center, Palo Alto, CA.

THE EFFECT OF SOLVENT POLARITY UPON ROTATIONAL BARRIERS IN NIKETHAMIDE

This is a report on a chemical property of nikethamide which could account, in part, for its widespread action on the central nervous system: the hindered internal rotation of the diethylamide group. In summary, dynamic nuclear magnetic resonance techniques were used to study the hindered internal rotation of the amide bond of the analeptic nikethamide. The rotatory motion of this bond was studied in a series of solvents of increasing polarity: $CDCl_3$, $CH_3(CH_2)_4OD$, CH_3CH_2OD, CH_3OD and D_2O. Motion about the amide bond was increasingly hindered in direct proportion to solvent polarity, correlating with enhanced hydrogen bond formation between nikethamide and the more polar solvent molecules. Diethylamide group motion would be expected to affect binding of the carbonyl oxygen to cholinergic receptor sites. The degree to which association to a receptor site can be affected by this rotatory motion may vary from 0 to 4 kcal/mole, the variability being entirely dependent upon the polarity of the binding site. An increase in rotamer lifetime, corresponding to a more polar environment, would be expected to enhance the kinetics of nikethamide association to the receptor site.

Bean, J. W. and *Nelson, D. J.*

Biochemical Pharmacology 33(13):2145-2149, 1984.

From the Chemistry Department, Clark University, Worcester, MA.

^{155}Eu AS A PROBE OF CHOLIN ACETYLCHOLINE RECEPTOI *MELANOGASTER* AND *TORPI*

For this study, $^{155}Eu^{3+}$ exch receptor proteins isolated from t cerebral ganglion of *Drosophila n* nuclear counting apparatus desig apparatus continuously monitors of two flow dialysis cells facilitati protein is dialyzed against buffer perturbed by the presence of nicc $^{155}Eu^{3+}$ displacement from the re related to the structure of the lig tonated pyridyl nitrogen atoms ir tonated forms of the molecules monium ion and carbamylcholi displaces bound $^{155}Eu^{3+}$ from the tetraethylammonium ion does, v choline does.

Bean, J. W., Rosenthal, L. S.,

Journal of the Less-Common M

From the Department of Chemi ter, MA; Department of Pharma Worcester.

ESR SPIN-TRAPPING STUD NOx/OLEFIN REACTIONS: OF THE APPARENTLY LON CIGARETTE SMOKE

Gas-phase cigarette smok carbon-centered free radicals. several variations of the electror use of spin traps in the solid stat gas phase. These gas-phase rad more than 5 min old, a result th of oxygen- and carbon-centere authors hypothesize that free cigarette smoke and exist in a *st* radicals can be formed involve oxide (which acts as a "radical Nitrogen dioxide can then react to produce the radicals that they the reactions of NO/air mixture most abundant species in smok

^{153}Eu AS A PROBE OF CHOLINERGIC LIGAND INTERACTIONS WITH ACETYLCHOLINE RECEPTOR PROTEINS ISOLATED FROM *DROSOPHILA MELANOGASTER* AND *TORPEDO CALIFORNICA*

For this study, $^{153}Eu^{3+}$ exchange experiments were performed on acetylcholine receptor proteins isolated from the electroplax tissue of *Torpedo californica* and the cerebral ganglion of *Drosophila melanogaster* utilizing a dual-chambered flow dialysis nuclear counting apparatus designed and constructed in the authors' laboratory. The apparatus continuously monitors $^{153}Eu^{3+}$ γ ray emission from the protein compartments of two flow dialysis cells facilitating the measurement of exchange half-lives. Receptor protein is dialyzed against buffer in the first cell, while in the second cell the receptor is perturbed by the presence of nicotinic ligand in the dialysate. Nicotinic ligands induce $^{153}Eu^{3+}$ displacement from the receptor proteins of both species in a manner directly related to the structure of the ligand. Nicotine and nikethamide molecules with protonated pyridyl nitrogen atoms induce $^{153}Eu^{3+}$ exchange significantly, while the deprotonated forms of the molecules do not effect exchange. Acetylcholine, tetraethylammonium ion and carbamylcholine all possess a quarternary nitrogen. Acetylcholine displaces bound $^{153}Eu^{3+}$ from the acetylcholine receptor proteins more readily than the tetraethylammonium ion does, which in turn induces exchange better than carbamylcholine does.

Bean, J. W., Rosenthal, L. S., *Nelson, D. J.*, and Wright, G. E.

Journal of the Less-Common Metals 94:367-374, 1983.

From the Department of Chemistry, Jeppson Laboratory, Clark University, Worcester, MA; Department of Pharmacology, University of Massachusetts Medical Center, Worcester.

ESR SPIN-TRAPPING STUDY OF THE RADICALS PRODUCED IN NOx/OLEFIN REACTIONS: A MECHANISM FOR THE PRODUCTION OF THE APPARENTLY LONG-LIVED RADICALS IN GAS-PHASE CIGARETTE SMOKE

Gas-phase cigarette smoke contains high concentratons of both oxygen- and carbon-centered free radicals. The investigators have detected these radicals using several variations of the electron spin resonance spin-trapping technique, including the use of spin traps in the solid state, to show that the radicals are trapped *directly* from the gas phase. These gas-phase radicals can still be trapped from gas-phase smoke that is more than 5 min old, a result that is clearly inconsistent with the highly reactive nature of oxygen- and carbon-centered radicals. To rationalize this apparent paradox, the authors hypothesize that free radicals are continuously produced and destroyed in cigarette smoke and exist in a *steady* state. They suggest that one mechanism by which radicals can be formed involves the slow oxidation of the relatively unreactive nitric oxide (which acts as a "radical reservoir") to the much more reactive nitrogen dioxide. Nitrogen dioxide can then react with a number of the species that are present in smoke to produce the radicals that they detect. The model used by the researchers consisted of the reactions of NO/air mixtures with unsaturated hydrocarbons. Isoprene is one of the most abundant species in smoke and is known to be very reactive toward NO_3; there-

fore, they have studied the nature of the radicals that can be spin trapped from gaseous mixtures of NO_2, isoprene and air. They found that the NO/air/isoprene model system gives essentially the same types of radicals (oxygen- and carbon-centered) as does cigarette smoke. They have also studied the gas-phase reactions of NO_2 with several small olefins and 1,3-butadiene and found evidence for peroxyl radical intermediates. In solution, NO_2 reacts with isoprene much faster than it does with the spin-trap phenyl-*tert*-butylnitrone (PBN). The researchers find that NO_2 oxidizes PBN to benzoyl *tert*-butyl nitroxide and propose a mechanism for this reaction.

Pryor, W. A., Tamura, M., and Church, D. F.

Journal of the American Chemical Society 106(18):5073-5079, 1984.

Other support: National Institutes of Health.

From the Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge.

NICOTINE AND PROSTAGLANDIN BIOFORMATION: AN *IN VITRO* STUDY WITH SPECIAL REFERENCE TO SOME TISSUES IN THE VASCULAR SYSTEM IN ANIMALS AND IN MAN

The effect of nicotine on the conversion of arachidonic acid (AA) to prostacyclin (PGI_2), primary prostaglandins (PGs) and thromboxane (Tx) in the rabbit, guinea pig, rat, and human vascular system *in vitro* was investigated. The effect of nicotine was also studied on a purified enzyme — prostaglandin endoperoxide synthetase (PES) — prepared from ram seminal vesicles. Results showed that prostacyclin production by aortic tissue from rabbit, guinea pig and rat — either spontaneous or following incubation with ^{14}C-AA — was inhibited dose-dependently by nicotine. The inhibition was competitive and localized to the enzyme, PES, that converts AA to PG endoperoxide. Nicotine did not affect the enzyme PGI_2 synthetase. It was found that in all species investigated, nicotine dose-dependently inhibited the formation of primary PGs by renal homogenates or microsomal fractions. The formation of TxB_2, in which the two enzymes PES and Tx synthetase are involved, in incubations of platelets with AA was completely unaffected by nicotine in all species. Also, experiments with the purified enzyme PES confirmed the inhibitory effect of nicotine. Overall, the data clearly demonstrate that nicotine inhibits PES in some but not all tissues in the vascular system in the investigated animals and in man. The possible implication of such a tissue difference in sensitivity to nicotine, for the relation between tobacco smoking and cardiovascular disease, is pointed out.

Alster, P. (*Wennmalm, Å.*)

Thesis, Stockholm, 1983.

Other support: The Swedish Tobacco Company, the Swedish Medical Research Council, Karolinska Institutet, and Stiftelsen Clas Groschinskys Minnesfond.

From the Department of Clinical Physiology, Karolinska Institutet, Huddinge University Hospital, Stockholm, Sweden.

EFFECT OF NICOTINE ON TH ACTIVITY AND THROMBOX

The effect of nicotine on the ane (TxB_2) in rabbit aorta and pla ment, rabbit aortic rings were inc incubation products were separ ternatively, the aortic rings were formation of platelet anti-aggreg somes were incubated with [^{14}C, t.l.c. In addition, rings of aorta w AA to labeled 6-keto-$PGF_{1\alpha}$ (the incubated in saline medium spon was dose-dependently inhibited b that platelet microsomes converte fected by nicotine. In summary, nicotine exists between cycloox demonstrate a tissue difference utilization of exogenous AA as su

Alster, P. and *Wennmalm, Å.*

British Journal of Pharmacology

Other support: Swedish Tobacco fond.

From the Department of Clinica dinge, Sweden.

VI. *Immunolog*

SUPPRESSOR T CELL ACTIV FERON

The interferons are a group c variety of immune responses. M suppressor T lymphocytes *in vitr* spleen cells. Suppression of huma here to determine whether human cyte IFN (IFNα) suppressed po ulin production by human periph doses of 200 to 350 U/ml. Resp dose-dependent manner; control studies, PBMC incubated with I cells that decreased pokeweed m duction by autologous cells by prevented by catalase, ascorbic

EFFECT OF NICOTINE ON THE FORMATION OF PROSTACYCLIN-LIKE ACTIVITY AND THROMBOXANE IN RABBIT AORTA AND PLATELETS

The effect of nicotine on the bioformation of prostacyclin (PGI_2) and of thromboxane (TxB_2) in rabbit aorta and platelets, respectively, was investigated. In one experiment, rabbit aortic rings were incubated with [^{14}C]-arachidonic acid ([^{14}C]-AA) and the incubation products were separated with thin layer chromatography (t.l.c.). Alternatively, the aortic rings were incubated without substrate and their spontaneous formation of platelet anti-aggregatory activity was measured. Rabbit platelet microsomes were incubated with [^{14}C]-AA and the products formed were separated with t.l.c. In addition, rings of aorta were found to be incapable of converting added [^{14}C]-AA to labeled 6-keto-$PGF_{1\alpha}$ (the stable hydrolysis product of PGI_2). Rings of aorta incubated in saline medium spontaneously formed PGI_2-like activity. This formation was dose-dependently inhibited by nicotine, with an I_{50} of about 10^{-4} M. It was also seen that platelet microsomes converted ^{14}C-AA to labeled TxB_2. This formation was unaffected by nicotine. In summary, it is concluded that a true difference in sensitivity to nicotine exists between cyclooxygenase in rabbit aorta and platelets. The data also demonstrate a tissue difference between rabbit aorta and platelets concerning their utilization of exogenous AA as substrate in the formation of platelet active compounds.

Alster, P. and *Wennmalm, Å.*

British Journal of Pharmacology 81:55-60, 1984.

Other support: Swedish Tobacco Company and Stiftelsen Clas Groschinskys Minnesfond.

From the Department of Clinical Physiology, Huddinge University Hospital, Huddinge, Sweden.

VI. *Immunology and Adaptive Mechanisms*

SUPPRESSOR T CELL ACTIVATION BY HUMAN LEUKOCYTE INTERFERON

The interferons are a group of proteins that inhibit viral replication and modulate a variety of immune responses. Murine fibroblast interferon (IFNβ) activates murine suppressor T lymphocytes *in vitro*, which suppress plaque-forming cell responses by spleen cells. Suppression of human *in vitro* immune responses by IFN was investigated here to determine whether human IFN also activates suppressor T cells. Human leukocyte IFN (IFNα) suppressed pokeweed mitogen-induced polyclonal immunoglobulin production by human peripheral blood mononuclear cells (PBMC) by 80–90% at doses of 200 to 350 U/ml. Responses by IFNα-treated PBMC were suppressed in a dose-dependent manner; control cultures had maximal responses on day 7. In other studies, PBMC incubated with 10,000 U/ml of IFNα contained activated suppressor cells that decreased pokeweed mitogen-stimulated polyclonal immunoglobulin production by autologous cells by 70–80%. Suppression mediated by these cells was prevented by catalase, ascorbic acid and 2-mercaptoethanol. Results from these and

related investigations indicate that IFNα activates suppressor T cells in human PBMC cultures, while the ability of catalase, 2-mercaptoethanol, and ascorbic acid to block suppression suggests that these suppressor T cells have certain similarities to IFNβ or α concanavalin A-activated murine suppressor T cells.

Schnaper, H. W., *Aune, T. M.* and Pierce, C. W.

The Journal of Immunology 131(5):2301-2306, 1983.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Departments of Pathology and Microbiology-Immunology, and Pediatrics, Washington University School of Medicine, St. Louis.

CHARACTERIZATION AND MECHANISM OF ACTION OF SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS)

Soluble immune response suppressor (SIRS), a product of concanavalin A-activated murine Lyt 2^+ T cells, which nonspecifically suppresses immune responses *in vitro*, is also produced by Lyt 2^+ T cells activated with fibroblast interferon (IFNβ). The SIRS suppressor pathway, as currently perceived, is summarized in this paper. To note, Lyt 2^+ T cells, activated by con A or IFNβ, release SIRS. The target of SIRS is the macrophage which activates or oxidizes SIRS through a peroxide-mediated reaction. Catalase blocks conversion of SIRS to $SIRS_m$ by consuming peroxide. Levamisole also prevents conversion of SIRS to $SIRS_m$ by blocking activation of SIRS by peroxide. The mechanism of $SIRS_m$-mediated inhibition of cell division appears to involve oxidation of cellular protein sulfhydryl groups which is time-dependent, proportional to the amount of $SIRS_m$ added and prevented by dithiothreitol or 2-mercaptoethanol. The applicability of this pathway to immunosuppression or to inhibition of cell division in general remains to be determined. The finding that IFNβ activates this pathway and that levamisole blocks suppression by the SIRS pathway suggests that it may be an important host mechanism for regulating both immune responses and cellular proliferation in general.

Aune, T. M. and Pierce, C. W.

In: Hadden, J. W. *et al.* (eds.): *Advances in Immunopharmacology 2*, New York: Pergamon Press, 1983, pp. 597-602.

Other support: National Institute of Allergy and Infectious Diseases and the National Science Foundation.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Department of Pathology and of Microbiology-Immunology, Washington University School of Medicine, St. Louis.

PROPERTIES OF THE SIRS SUPPRESSOR PATHWAY

In this study of the properties of the SIRS suppressor pathway, it was found that the SIRS suppressor pathway is initiated by activation of Ly 2^+ T lymphocytes by either con A or IFNβ. SIRS is a protein which has been purified and exists as two species with mol. wts. of 14,000 and 21,500. The target of SIRS is the macrophage and macrophages appear to oxidize or activate SIRS in a peroxide dependent process. Catalase blocks SIRS or IFNβ action by consuming H_2O_2 and levamisole blocks SIRS

or IFNB by preventing activati
block SIRS or IFNβ action in
$SIRS_m$ is a potent inhibitor of
neoplastic cells. The mechanism
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Aune, T. H. and Pierce, C. W.

In: *13th International Cancer C*
Alan R. Liss, Inc., 1983, pp. 3

Other support: National Scien

From the Department of Pathol
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Schnaper, H. W., Pierce, C. W

The Journal of Immunology 132

Other support: Monsanto Com

From the Department of Pathol
St. Louis, and the Departments
School of Medicine, St. Louis.

or IFNB by preventing activation or oxidation of SIRS by H_2O_2. Other agents which block SIRS or IFNβ action include electron donors which can inactivate $SIRS_m$. $SIRS_m$ is a potent inhibitor of immune responses and proliferation of normal and neoplastic cells. The mechanism of $SIRS_m$-mediated inhibition of proliferation appears to involve oxidation or modification of protein sulfhydryls. As of now, both IFNβ and levamisole have been found to affect a wide variety of cellular processes. The involvement of both of these substances in the SIRS pathway suggests that this pathway may be an important host mechanism for regulating both immune responses and cellular proliferation in general.

Aune, T. H. and Pierce, C. W.

In: *13th International Cancer Congress, Part B: Biology of Cancer (1)*, New York: Alan R. Liss, Inc., 1983, pp. 335-344.

Other support: National Science Foundation and the National Institutes of Health.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Department of Pathology and of Microbiology-Immunology, Washington University School of Medicine, St. Louis.

IDENTIFICATION AND INITIAL CHARACTERIZATION OF CONCAVALIN A- AND INTERFERON-INDUCED HUMAN SUPPRESSOR FACTORS: EVIDENCE FOR A HUMAN EQUIVALENT OF MURINE SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS)

Antigen-nonspecific suppression in both murine and human systems may be mediated through several divergent pathways, through an ultimate common pathway, or through a combination of these two possibilities. In the paper presented here, it is noted that human suppressor T cells activated by leukocyte interferon have properties similar to murine suppressor cells activated by interferon or by concanavalin A. Murine suppressor cells release a soluble mediator, soluble immune response suppressor (SIRS), which accounts, at least in part, for suppressive activity in murine systems. To compare and contrast murine and human suppressor pathways, these investigators evaluated the suppression of human polyclonal plaque-forming cell responses by concanavalin A, by leukocyte interferon, and by immune interferon, or by suppressor cells activated by these agents. In each instance, suppressor cells released suppressor factors with similar properties that were indistinguishable from murine SIRS. As with murine SIRS, suppression by each of these human factors was inhibited by 2-mercaptoethanol, ascorbic acid, catalase, or levamisole. Significantly, the similarities between these human suppressor factors and murine SIRS show the existence of a human SIRS pathway.

Schnaper, H. W., Pierce, C. W. and *Aune, T. M.*

The Journal of Immunology 132(5):2429-2435, 1984.

Other support: Monsanto Company.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Departments of Pathology and of Pediatrics, Washington University School of Medicine, St. Louis.

MECHANISM OF SIRS ACTION AT THE CELLULAR AND BIOCHEMICAL LEVEL

In this comprehensive paper on SIRS, sections are devoted to T Cell Hybridomas Producing SIRS; Purification of SIRS; Mechanisms of Action of SIRS as regards: Inhibition of Immune Function, Inhibition of Cell Division, $SIRS_{ox}$-Mediated Cellular Protein Sulfhydryl Group Loss, and $SIRS_{ox}$-Mediated Inhibition of Microtubule Function. The discussion section of this paper points out that the SIRS suppressor pathway is initiated by activation of murine T lymphocytes with either concanavalin A or interferon β. Ly 2+ T lymphocytes release SIRS whose target is the macrophage. Macrophages appear to oxidize SIRS to $SIRS_{ox}$ in an H_2O_2-dependent process and $SIRS_{ox}$ is directly reponsible for inhibition of *in vitro* immune responses. Catalase prevents SIRS or IFNβ-mediated inhibition by competing with SIRS for H_2O_2. Similarly, the immunoenhancing drug, levamisole, inhibits IFNβ or SIRS-mediated suppression by inhibiting activation of SIRS by either macrophages or H_2O_2. Other observations considered in this discussion include: (1) SIRS/$SIRS_{ox}$ appears to be a general inhibitor of cellular proliferation. (2) $SIRS_{ox}$ appears to inhibit cell division by modifying certain protein sulfhydryl groups. (3) Certain features of $SIRS_{ox}$-catalyzed oxidation of protein sulfhydryl groups are similar to the enzymatic properties of sulfhydryl oxidase isolated from milk. (4) At this point it is not certain whether or to what extent the SIRS pathway participates in host regulation of immune responsiveness or cell division.

Aune, T. M. and Pierce, C. W.

In: *Lymphokines, Vol. 9*, New York: Academic Press, 1984, pp. 257-277.

Other support: National Science Foundation and the National Institutes of Health.

From the Department of Pathology and Laboratory Medicine, The Jewish Hospital of St. Louis, and the Department of Pathology and Microbiology-Immunology, Washington University School of Medicine, St. Louis.

CATHEPSIN G IN HUMAN MONONUCLEAR PHAGOCYTES: COMPARISONS BETWEEN MONOCYTES AND U937 MONOCYTE-LIKE CELLS

Data demonstrating that U937 cells contain cathepsin G-like activity are presented in this report. U937 cells contain approximately 10μg of cathepsin G-like activity per 10^7 cells, about 25% of the cathepsin G activity in human neutrophils. Normal monocytes have minimal cathepsin G-like activity (approximately 0.1μg per 10^7 cells). The cathepsin G-like activity of U937 cells appears to be due to an enzyme that is the same as cathepsin G by several criteria: (1) it is a serine proteinase with activity like cathepsin G against a synthetic chymotrypsin substrate, succinyl-ala-ala-pro-phe-p-nitroanilide; (2) the proteolytic fragments it releases from fibronectin match those released by cathepsin G; (3) like cathepsin G, it can be purified by sequential Trasylol-Sepharose affinity chromatography and carboxymethyl-Sephadex ion exchange chromatography; (4) its amino acid composition and migration on SDS-polyacrylamide gel electrophoresis are indistinguishable from cathepsin G; and (5) it binds with antiserum raised to cathepsin G. The presence of cathepsin G in U937 cells, in much higher concentration than in normal monocytes, indicates either that the content of cathepsin G in monocytes decreases markedly during monocyte differentiation or

that U937 cells differ from n
this neutral proteinase.

Senior, R. M. and *Campbell,*

The Journal of Immunology 1

Other support: U. S. Public

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ANTIGENIC ANALYSIS O
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Strauss, L. C., Stuart, R. K.

Blood 61(6):1222-1231, 1983.

Other support: National Inst

From the Department of Onc
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that U937 cells differ from normal immature monocytes with respect to synthesis of this neutral proteinase.

Senior, R. M. and *Campbell, E. J.*

The Journal of Immunology 132(5):2547-2551, 1984.

Other support: U. S. Public Health Service.

From the Respiratory and Critical Care Division, Department of Medicine, Jewish Hospital at Washington University Medical Center, St. Louis.

ANTIGENIC ANALYSIS OF HEMATOPOIESIS. I. EXPRESSION OF THE MY-1 GRANULOCYTE SURFACE ANTIGEN ON HUMAN MARROW CELLS AND LEUKEMIC CELL LINES

Plasma membrane components appear to mediate many granulocyte functions. For example, cell surface receptors for the Fc region of immunoglobulin and for the third component of complement play roles in granulocyte phagocytosis and antibody-mediated cellular cytotoxicity. These receptors are not granulocyte-specific, however, since they are expressed on cells of other lineages. The existence of human neutrophil-specific antigens has been inferred from studies of immune granulo-cytopenia. In the study reported here, five monoclonal antibodies that identify the My-1 human granulocyte surface antigen were not reactive with other peripheral blood cells. These antibodies affected complement-dependent cytolysis of a large fraction of normal human marrow leukocytes. This My-1-positive marrow cell population consisted of morphologically identifiable granulocytic precursor cells. Colony-forming cells of the granulocyte-monocyte lineage (CFC-GM) did not express My-1, suggesting that the My-1 antigen is expressed later in normal granulocytic maturation. However, these antibodies did react with myeloid leukemia cell lines. The significance and potential utility of these probes for the understanding of granulopoietic differentiation is discussed.

Strauss, L. C., Stuart, R. K., and *Civin, C. I.*

Blood 61(6):1222-1231, 1983.

Other support: National Institutes of Health and the Heart of Variety Fund.

From the Department of Oncology, Divisions of Pediatric Oncology and Experimental Hematology, Johns Hopkins University School of Medicine, Baltimore.

ANTIGENIC ANALYSIS OF HEMATOPOIESIS: II. EXPRESSION OF HUMAN NEUTROPHIL ANTIGENS ON NORMAL AND LEUKEMIC MARROW CELLS

Hybridoma-derived monoclonal antibodies (McAb) specifically reactive with lymphocyte cell surface molecules have been of great value in the analysis of lymphocyte differentiation and lymphoid neoplasia. McAb reactive with human neutrophils have been developed, and antibodies against the My-1 human granulocyte antigen have been shown to react with morphologically identifiable neutrophil precursors, but not with colony-forming cells of the granulocyte-monocyte lineage (CFC-GM). In the

work reported here, the binding of five antineutrophil monoclonal antibodies AHN-1, -2, -3, -7, and -8, to normal and leukemic bone marrow cells was studied. AHN-7 bound to many granulocytic precursors, particularly myelocytes, and both lymphoid and blast cells in normal marrow, and to most but not all granulocyte-macrophage progenitors (CFC-GM). AHN-8 bound only to late (band and segmented) neutrophilic cells and not to CFC-GM. AHN-1, -2, -3 bound to morphologically identifiable neutrophil precursors, but not to (day-14) CFC-GM. Approximately half of non-lymphoid leukemia specimens were positive with AHN-1 or AHN-7; by contrast, lymphoid leukemia specimens were rarely positive. AHN-8 was rarely found on leukemia cells. These antineutrophil antibodies appear to detect distinct granulopoietic subsets and may be useful in the analysis of hematologic differentiation and in the subclassification of leukemias.

Strauss, L. C., Skunitz, K. M., August, J. T., and *Civin, C. I.*

Blood 63(3):574-578, 1984.

Other support: National Institutes of Health, Heart of Variety Fund and the Johnson & Johnson Company.

From the Department of Oncology, Division of Pediatric Oncology, and the Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore.

ANTIGENIC ANALYSIS OF HEMATOPOIESIS. III. A HEMATOPOIETIC PROGENITOR CELL SURFACE ANTIGEN DEFINED BY A MONOCLONAL ANTIBODY RAISED AGAINST KG-1A CELLS.

In this study, the KG-1a human leukemic cell line was used as an immunogen in an attempt to produce antibodies against human blast cell-surface antigens. The KG-1 myeloblastic leukemic cell line was derived from a patient with nonlymphocytic leukemia and the KG-1a cell line arose from it as a spontaneous tissue culture variant. As reported here, the anti-My-10 mouse monoclonal antibody was raised against the immature human lyeloid cell line KG-1a and was selected for nonreactivity with mature human granulocytes. Anti-My-10 immunoprecipitated a KG-1a cell surface protein with an apparent Mr of approximately 115 kD. The authors describe the binding of this antibody to human hematopoietic cell types and show that My-10 is expressed specifically on immature normal human marrow cells, including hematopoietic progenitor cells. My-10 is also expressed by leukemic marrow cells from a subpopulation of patients. Thus, this antibody allows the identification and purification of hematopoietic progenitor cells from normal human marrow and the subclassification of leukemia.

Civin, C. I. et al.

The Journal of Immunology 133(1):157-165, 1984.

Other support: National Institutes of Health and the Heart of Variety Fund.

From the Johns Hopkins Oncology Center, Divisions of Pediatric Oncology and Cell Structure and Function; and the Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore.

RAPID, EFFICIENT CLON
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RAPID, EFFICIENT CLONING OF MURINE HYBRIDOMA CELLS IN LOW GELATION TEMPERATURE AGAROSE

This paper describes a simple method for cloning of hybridomas that is rapid, reliable and efficient in terms of cost, time and cloned hybridoma recovery. The method utilizes highly-purified, extremely low-gelation temperature agarose that can be used for extended periods of time at room temperature without gelation. To be specific, the use of ultra-low gelation temperature agarose allowed the investigators to avoid placing a water bath, a potential source of microbial contamination, in their laminar flow hood. More important, cells never needed to be exposed to potentially toxic temperatures, >37°C. In addition, several lots of this agarose have been tried and were not toxic to hybridoma cells. Cloning time and efforts were reduced by avoiding the need for constant manipulations to maintain medium temperature above 37°C to prevent gelation of conventional agarose. Five or more hybridomas can easily be cloned in an hour using this method. In this study, cloning efficiency varied from hybridoma to hybridoma. However, it was found that clones which were proliferating rapidly and were highly viable routinely had cloning efficiencies higher, by orders of magnitude, than clones which were growing poorly in liquid cultures.

Civin, C. I. and Banquerigo, M. L.

Journal of Immunological Methods 61:1-8, 1983.

Other support: National Institutes of Health, the Blood Systems, and the Heart of Variety Fund

From the Department of Oncology, Division of Pediatric Oncology, Johns Hopkins University School of Medicine, Baltimore.

A SYNTHETIC PEPTIDE INDUCES A NEW ANTI-DEXTRAN IDIOTYPE

One of the major factors confounding attempts to make serologic and molecular correlations is the complexity of the idiotypic determinants defined by conventional serologic techniques. There has recently been a new approach to this problem, though, since peptides which mimic the primary sequences of numerous antigens have been used to generate antibodies which bind both peptide and the whole molecules from which the peptide was derived. In the summary of the paper presented here, it is shown that the J_H1 peptide has been synthesized and coupled to keyhole limpet hemocyanin for use as an immunogen. A serum from an animal hyperimmunized with this immunogen demonstrated binding to both peptide and native immunoglobulin. Furthermore, this serum seems to define an idiotypic determinant nearly unique to $\alpha(1\rightarrow3)$ dextran-binding immunoglobulins. Synthetic peptide immunogens may be a useful tool in probing the molecular basis of idiotypy.

Seiden, M. V., *Clevinger, B.*, Srouji, T., Davie, J. M., McMillan, S., and Lerner, R.

Annales d'Immunologie (Inst. Pasteur) 135:77-82, 1984.

Other support: National Institutes of Health.

From the Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis; Department of Biomedical Science, Washington University School of Dental Medicine, St. Louis, and Department of Molecular Biology, the Research Institute of Scripps Clinic, La Jolla, CA.

CHEMICAL SYNTHESIS OF IDIOTOPES: EVIDENCE THAT ANTISERA TO THE SAME JH_1 PEPTIDE DETECT MULTIPLE BINDING SITE-ASSOCIATED IDIOTOPES

More than 20 years ago, Kunkel, H. G., Mannik, M. and Williams, R. C. (1963) showed that the structural diversity expressed by variable region domains could be detected by antisera. These variable region antigenic determinants, called idiotopes, provide simple and highly specific ways of identifying and comparing variable regions and have been widely used in immunological research. In addition to providing useful markers for investigating variable region structure and function, idiotypes may provide an important means of immune regulation. To better understand the molecular basis of idiotypy, the investigators have generated several site-specific antisera through immunization of animals with synthetic peptides corresponding to the (JH_1) heavy chain joining segment 1 of the mouse heavy chain variable (V_H) region. These antipeptide sera identify several idiotypic determinants present on intact hybridoma and myeloma immunoglobulins. Expression of at least three of these idiotopes is correlated with the antigen specificity of the family of immunoglobulins bearing the determinant. Use of synthetic peptides may prove a powerful technique in the generation of molecularly defined antiidiotypic reagents.

Seiden, M. V., *Clevinger, B.*, McMillan, S., Srouji, A., Lerner, R., and Davie, J. M.

Journal of Experimental Medicine 159:1338-1350, 1984.

Other support: U. S. Public Health Service.

From the Department of Microbiology and Immunology, School of Medicine, and Division of Biomedical Science, School of Dental Medicine, Washington University, St. Louis, and the Department of Molecular Biology, Research Institute of the Scripps Clinic, La Jolla, CA.

RADIOENZYMATIC ASSAY FOR MEASUREMENT OF TISSUE CONCENTRATIONS OF HISTAMINE: ADAPTATION TO CORRECT FOR ADHERENCE OF HISTAMINE TO MECHANICAL HOMOGENIZERS

Measurements of histamine concentrations in tissue have important experimental applications. Because adherence of histamine to glass is well-known, the present investigators tested for its adherence to a mechanical homogenizer commonly used in the extraction of histamine from tissue samples. During 60 sec of homogenization, 15% to 71% of the histamine originally present in the samples "disappeared" and the reason for the disappearance was reversible binding of histamine to the homogenizer. Adding trace amounts of [^{3}C] histamine to each sample before homogenization and measuring the disappearance of radioactivity during homogenization permitted correction for binding to the homogenizer. This technique for correction was validated by the measurement of endogenous concentrations of histamine in the tracheal posterior membranes of six dogs (range of mean concentrations: 0.63 to 1.51 ng/mg wet weight) followed by the measurement of known amounts of exogenous histamine added before homogenization to tracheal tissue samples from the same dogs. In the latter samples, 96 ± 13% (mean ± SEM) of the histamine added was measured by this technique. The authors conclude that binding of histamine to mechanical homogenizers may be an

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Brown, J. K., Frey, M. J., Reed

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Other support: U. S. Public He

From the Cardiovascular Researc
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Clinical Immunology and Imm

Other support: National Institu
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From the Departments of Pedi
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Brown, J. K., Frey, M. J., Reed, B. R., Leff, A. R., Shields, R., and *Gold, W. M.*

The Journal of Allergy and Clinical Immunology 73(4):473-478, 1984.

Other support: U. S. Public Health Service.

From the Cardiovascular Research Institute and the Department of Medicine, University of California, San Francisco, and Respiratory Care Section, Department of Medicine, Veterans Administration Medical Center, San Francisco.

IMBALANCES IN SUBSETS OF T LYMPHOCYTES IN AN INBRED PEDIGREE WITH OMENN'S SYNDROME

This report describes the immunologic function and immunoregulatory subsets of T lymphocytes in two affected infants and 109 healthy family members of the same pedigree originally described by Omenn. Eighteen homozygotes in this pedigree had previously died from infection at less than six months of age. Both of the infants described here displayed normal numbers of peripheral blood T (E-rosette) lymphocytes, poor mitogen reactivity of lymphocytes, normal mixed lymphocyte culture reactivity, a paucity of circulating B cells, variable hypogammaglobulinemia, and elevated serum IgE concentrations. At four months of age, one infant (boy) had 95% T3+ (total T) peripheral blood lymphocytes, 41% T4+ (helper T) lymphocytes, and 64% T8+ (suppressor T) lymphocytes; at four months of age the other infant (girl) had 43% T3+, 43% T4+, 19% T8+ lymphocytes, and 18% T6+ (stage II thymocyte) lymphocytes. Age-matched controls had values 49% for T3+, 37% for T4+, 13% for T8+, and <1% T6+. Functional lymphocyte suppression assayed by the reverse hemolytic plaque assay indicated that the infant girl's lymphocytes suppressed 75% of the immunoglobulin produced by normal lymphocytes. The 109 healthy family members (containing many obligate heterozygotes) were analyzed for distributions of T-lymphocyte subsets and compared with 37 age-matched controls. Based on these results, it was concluded that the immunodeficiency in Omenn's syndrome is due to deregulation of T-lymphocyte subsets, appearance of immature T cells in the peripheral blood, functional T-cell suppression of immunoglobulin production, and reduced B-cell populations. An abnormal distribution of the percentage of T4- and T8-positive cells, which exists in an extraordinary number of family members, may serve as a phenotypic lymphocyte marker and thereby aid in the identification of the heterozygous state.

Karol, R. A., Eng, J., Cooper, J. B., Dennison, D. K., Sawyer, M. K., *Lawrence, E. C.*, Marcus, D. M., and Shearer, W. T.

Clinical Immunology and Immunopathology 27:412-427, 1983.

Other support: National Institutes of Health, General Clinical Research Center, John P. McGovern Foundation, and the Texas Children's Hospital.

From the Departments of Pediatrics, Medicine, and Microbiology and Immunology, Baylor College of Medicine, and the Allergy and Immunology Service, Texas Children's Hospital, Houston, and the Department of Pediatrics, University of Texas Health Science Center, Dallas.

INEFFECTIVE IMMUNOGLOBULIN SECRETION IN RESPONSE TO POKEWEED MITOGEN IN SARCOIDOSIS

Sarcoidosis is a systemic granulomatous disease of unknown cause associated with alterations in both cellular and humoral immune functions. Recently, monocytes which suppress pokeweed mitogen (PWM)-induced specific antibody formation have been reported in sarcoidosis. The purpose of the current study was to evaluate whether suppressor monocytes might also be operative for polyclonal immunoglobulin (Ig) secretion in this disease. Accordingly, peripheral blood mononuclear cells (MNL) from sarcoid patients and normal control subjects were cultured with PWM for six days and the total number of Ig-secreting cells (Ig-SC) determined with a reverse hemolytic plaque assay. While normal MNL responded to PWM with a 10-fold or greater increase in Ig-SC, sarcoid MNL failed to respond to PWM. Sarcoid MNL contained greater percentages of monocytes than normals (44.8 ± 2.0% vs. 30.4 ± 1.4%, $p < 0.001$) and there was a negative correlation between the magnitude of the response to PWM and the percentage of MNL-monocytes. However, prior removal of monocytes improved the responsiveness to PWM in only four patients. Excessive suppressor monocyte activity in co-culture studies could be identified in only these same four patients. Thus, neither a relative monocytosis, while present in sarcoidosis, nor excessive suppressor monocyte activity may entirely account for the hyporesponsiveness to PWM.

Lawrence, E. C. et al.

In: Chretien, J., Marsac, J., and Saltiel, J. C. (eds.): *Sarcoidosis*, New York: Pergamon Press, 1981, pp. 98-102.

Other support: Gulf Oil Foundation and the American Lung Association.

From The Rockwell-Keough Pulmonary Immunology Laboratory and the General Clinical Research Center of the Methodist Hospital, and the Department of Medicine, Baylor College of Medicine, Houston.

CORRELATION OF DISEASE ACTIVITY IN SARCOIDOSIS WITH SERUM ANGIOTENSIN CONVERTING ENZYME, ^{67}GALLIUM LUNG SCANNING AND BRONCHOALVEOLAR LAVAGE

As stated in this paper, sarcoidosis is a granulomatous disease of unknown cause which is often treated with corticosteroids to relieve symptoms and to suppress inflammatory lung involvement. Gallium-67 (^{67}Ga) lung scanning, serum angiotensin-converting enzyme (SACE), and bronchoalveolar lavage (BAL) have been proposed as useful techniques to be followed in the assessment of disease activity and response to therapy in sarcoidosis. The purpose of the present study was to compare these various procedures with clinical assessments of disease activity in 87 studies performed over an 18-month period on 26 patients with sarcoidosis. While there were strong statistical associations between BAL, ^{67}Ga lung scanning, SACE levels and clinical assessments of disease activity, each parameter was useful in different ways. Thus, greater than 20% BAL-lymphocytes was associated with disease activity, whereas less than 3000 BAL-IgG secreting cells/10^6 BAL lymphocytes was always indicative of inactive disease. By contrast, SACE levels of 750 U/ml were always associated with disease activity. Quantitation of ^{67}Ga lung scanning correlated very well with clinical assessments of disease activity in both treated and untreated patients, with only rare false positive or false negative results. No single parameter may be infallible in the assess-

ment of disease activity in sarco
scanning and BAL analysis may

Lawrence, E. C. et al.

In: Cheretien, J., Marsac, J., ar

Pergamon Press, 1981, pp. 430-

Other support: Gulf Oil Found

From Rockwell-Keough Pulmor
Research Center of the Method
College of Medicine, Houston.

GANGLIOSIDES OF HUMA

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tant differences between the g
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Westrick, M. A., Lee, W. M

Biochimica et Biophysica Act

Other support: Cancer Resea

From the Cancer Research L
Pharmaceutical Chemistry, U

GANGLIOSIDES OF HUM
HAIRY CELLS

Gangliosides are an imp
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and structural characterizati
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ment of disease activity in sarcoidosis but determination of SACE levels, ^{67}Ga lung scanning and BAL analysis may be complementary toward this goal.

Lawrence, E. C. et al.

In: Cheretien, J., Marsac, J., and Saltiel, J. C. (eds.): *Sarcoidosis*, New York: Pergamon Press, 1981, pp. 430-433.

Other support: Gulf Oil Foundation and the American Lung Association.

From Rockwell-Keough Pulmonary Immunology Laboratory and the General Clinical Research Center of the Methodist Hospital, and the Department of Medicine, Baylor College of Medicine, Houston.

GANGLIOSIDES OF HUMAN ACUTE LEUKEMIA CELLS

In an attempt to gain more information on the gangliosides present in acute leukemia cells, these investigators characterized the gangliosides from cells of eight patients with different forms of acute leukemia (four lymphoblastic, four nonlymphoblastic) by thin-layer chromatography and high-performance liquid chromatography combined with glycosidase treatment. Their analysis indicated both quantitative and qualitative differences between the gangliosides of acute leukemia and those of normal leukocytes: (1) the absolute amount of ganglioside was decreased in the acute leukemia cells; (2) in general, acute leukemias had a more simplified ganglioside pattern in that they contained a greater proportion of the short-chain ganglioside, II^{3}NeuAc-LacCer (GM3); (3) all of the acute leukemia cells contained reduced quantities of the ganglioside *N*-acetylneuraminosyllactotriaosylceramide, a compound previously found only in normal leukocytes, and (4) a disialylated ganglioside, II^3(NeuAc)$_2$-LacCer (GD3), which is not found in normal leukocytes, was isolated from the cells of one patient with acute nonlymphoblastic leukemia. These findings demonstrate important differences between the gangliosides of acute leukemia cells and normal leukocytes.

Westrick, M. A., Lee, W. M. F., Goff, B., and *Macher, B. A.*

Biochimica et Biophysica Acta 750:141-148, 1983.

Other support: Cancer Research Funds of the University of California.

From the Cancer Research Institute, Department of Medicine and Department of Pharmaceutical Chemistry, University of California, San Francisco.

GANGLIOSIDES OF HUMAN CHRONIC LYMPHOCYTIC LEUKEMIA AND HAIRY CELLS

Gangliosides are an important subclass of glycosphingolipids which contain sialic acid. The research efforts of this laboratory have been focused on the quantification and structural characterization of neutral glycosphingolipids and gangliosides from human leukocytes and leukemia cells. In the study reported here, gangliosides were purified from the cells of two patients with hairy cell leukemia and one patient with

chronic lymphocytic leukemia. Quantification of these compounds showed that these cells contain only 5-15% of the amount of lipid-bound sialic acid (gangliosides) per cell as normal lymphocytes. Structural characterization by gas-liquid chromatography, glycosidase treatment and high-performance liquid chromatography demonstrated that the major gangliosides of these leukemia cells were of the lactosyl type. Hairy cells contained monosialyl-lactosylceramide (II^{3}NeuAc-LacCer); whereas chronic lymphocytic leukemia cells contained both monosialyl and disialyl lactosylceramide [II^3(NeuAc)$_2$-LacCer]. Chronic lymphocytic leukemia cells contained lesser amounts of three other gangliosides of the neolacto or lacto series as determined by endo-β-galactosidase treatment. None of these leukemia cells contained detectable quantities of NeuAc-LcOse$_4$Cer, a ganglioside found in normal leukocytes.

Goff, B. A., Lee, W. M. F., Westrick, M. A., and *Macher, B. A.*

European Journal of Biochemistry 130:553-557, 1983.

Other support: National Institutes of Health and the National Cancer Institute.

From the Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco.

ISOLATION AND CHARACTERIZATION OF GANGLIOSIDES FROM CHRONIC MYELOGENOUS LEUKEMIA CELLS

The purpose of this study was to isolate and structurally characterize the major chronic myelogenous leukemia (CML) gangliosides and compare them to the gangliosides of normal neutrophils. As presented here, gangliosides isolated from the cells of three patients with CML were purified by Folch partitioning, diethylaminoethyl Sephadex, Florisil (acetylated gangliosides), and silicic acid chromatography and were structurally analyzed using thin-layer and gas-liquid chromatography, methylation analysis, enzyme degradation, and high-performance liquid chromatography. With these methods, the major gangliosides isolated were II^3-α-N-acetylneuraminosyllactosylceramide, IV^3-α-acetylneuraminosyl-neolactotetraosylceramide (sialosylparagloboside), and a ganglioside with the following structure: NeuAcα2→3(Galβ1→4GlcNacβ1→3)$_2$Galβ1→4Glcβ1→1Cer. This ganglioside has previously been characterized as an "i" active compound. It was also found that, like normal neutrophils, CML cells contain monosialogangliasides that belong to the lactosyl and neolacto family. However, this study shows that CML cells differed from normal neutrophils in that they contained less total ganglioside, and their major ganglioside species is II^3-α-N-acetylneuraminosyllactosylceramide. Difference between gangliosides of CML and acute nonlymphoblastic leukemias are discussed.

Westrick, M. A., Lee, W. M. F., and *Macher, B. A.*

Cancer Research 43:5890-5894, 1983.

Other support: National Cancer Institute.

From the Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco.

ASSOCIATION OF GANGLIOSIDE AND SENDAI VIRUS: REQUIRE VIRAL FUSION

In this study, a method is descri in particular antibodies and their fr The protein-ganglioside conjugate i separated from free protein by mole versibly transfer from the micelle to be identified as a new surface antige has been demonstrated with three b gate has been transferred to humar glutinated with goat anti-rabbit Ig H2K^k have been shown to adhere express H2K^k-antigen. Mouse mon associate with Sendai virus and co hemolyze desialylated human ery these investigators demonstrated th for viral binding, appears also to be eliminates hemolysis and fusion, virus.

Heath, T. D., Martin, F. J. and *M*

Experimental Cell Research 149:16

Other support: National Cancer In

From the Cancer Research Institu University of California, San Fran

DIFFERENTIAL EXPRESSION LEUKOCYTES AND LEUKEMI

Earlier analyses of the glycos mia cells have shown that these c but only one disialoganglioside, (leukocytes and the cells of 25 pati the presence of disialoganglio neuraminosyllactosylceramide (G chromatographs with an anti-G_{D3} cells tested, acute leukemia cells v and normal neutrophils did not apparent within the acute myelo leukemia cells stained more inten characteristics. All lymphocytic l this ganglioside could not be ganglioside extract from the cells for G_{D3} immunostaining. These re myelogenous leukemia cells are leukemia cells on the basis of G_{D}

Siddiqui, B., Buehler, J., DeGr

ASSOCIATION OF GANGLIOSIDE-PROTEIN CONJUGATES INTO CELL AND SENDAI VIRUS: REQUIREMENT FOR THE HN SUBUNIT IN VIRAL FUSION

In this study, a method is described for preparing a covalent conjugate of proteins, in particular antibodies and their fragments, with gangliosides in the micellar form. The protein-ganglioside conjugate is associated with ganglioside micelles and can be separated from free protein by molecular sieve chromatography. Conjugates can irreversibly transfer from the micelle to a cell membrane of choice, and the protein portion be identified as a new surface antigen. The successful application of this methodology has been demonstrated with three biological systems. Rabbit IgG-ganglioside conjugate has been transferred to human or sheep erythrocytes, which have been hemagglutinated with goat anti-rabbit IgG. Erythrocytes modified with ganglioside-anti-H2K^{k} have been shown to adhere to monolayers of L929 mouse fibroblasts which express H2K^{k}-antigen. Mouse monoclonal antiglycophorin ganglioside conjugate can associate with Sendai virus and confer upon the virus the ability to agglutinate and hemolyze desialylated human erythrocytes. Using the antiglycophorin conjugate, these investigators demonstrated that the HN subunit, which is normally responsible for viral binding, appears also to be essential for fusion activity because its destruction eliminates hemolysis and fusion, but not agglutination, by the conjugate-modified virus.

Heath, T. D., Martin, F. J. and *Macher, B. A.*

Experimental Cell Research 149:163-175, 1983.

Other support: National Cancer Institute and the National Institutes of Health.

From the Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco.

DIFFERENTIAL EXPRESSION OF GANGLIOSIDE G_{D3} BY HUMAN LEUKOCYTES AND LEUKEMIA CELLS

Earlier analyses of the glycosphingolipids of normal human leukocytes and leukemia cells have shown that these cells contain several types of monosialogangliosides but only one disialoganglioside, G_{D3}. In the present study, gangliosides from normal leukocytes and the cells of 25 patients with acute and chronic leukemia were tested for the presence of disialoganglioside II^3-α-*N*-acetylneuraminosyl-α2→8-*N*-acetylneuraminosyllactosylceramide (G_{D3}). G_{D3} was detected by immunostaining thin-layer chromatographs with an anti-G_{D3} monoclonal antibody (AbR_{24}). Among the myeloid cells tested, acute leukemia cells were positive for G_{D3}, whereas chronic leukemia cells and normal neutrophils did not have detectable G_{D3}. A range of G_{D3} reactivity was apparent within the acute myeloid leukemia cells; gangliosides from pure myeloid leukemia cells stained more intensely than those from leukemia cells with monocytic characteristics. All lymphocytic leukemia cells (chronic and acute) contained G_{D3}, but this ganglioside could not be detected in extracts from normal lymphocytes. A ganglioside extract from the cells of a patient with hairy cell leukemia was also positive for G_{D3} immunostaining. These results demonstrate that normal leukocytes and chronic myelogenous leukemia cells are distinguished from other lymphoid and nonlymphoid leukemia cells on the basis of G_{D3} ganglioside expression.

Siddiqui, B., Buehler, J., DeGregorio, M. W., and *Macher, B. A.*

Cancer Research 44:5262-5265, 1984.

Other support: National Cancer Institute and Louis R. Lurie Foundation.

From the Gastrointestinal Research Laboratory, Veterans Administration Hospital, San Francisco; Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco; and Children's Cancer Research Institute, Pacific Medical Center, San Francisco.

INHIBITION OF MITOGEN- AND ANTIGEN-INDUCED LYMPHOCYTE ACTIVATION BY HUMAN LEUKEMIA CELL GANGLIOSIDES

This study demonstrated that gangliosides prepared from human leukemia cells inhibit mitogen and antigen activation of human lymphocytes. Furthermore, the investigators have analyzed the effect of purified and structurally defined gangliosides on lymphocyte blastogenesis. These analyses allow them to conclude that: (a) gangliosides of human leukemia cells, when added to *in vitro* assay systems in concentrations known to occur in the serum of humans with cancers, suppress lymphocyte activation; (b) each purified ganglioside suppressed blastogenesis to a similar extent; and (c) sialic acid is essential for maximal immunosuppression. The three gangliosides used in this study are also known to be components of normal cells and therefore should not be considered leukemia-associated components. However, the concentration of these compounds is known to be elevated in the serum of patients with cancers and, therefore, may contribute to a reduced immune response in some cancer patients.

Gonwa, T. A., Westrick, M. A., and *Macher, B. A.*

Cancer Research 44:3467-3470, 1984.

Other support: National Cancer Institute, U. S. Public Health Service, and National Research Service Awards.

From the Department of Medicine, University of Iowa, Iowa City, and Cancer Research Institute and the Department of Pharmaceutical Chemistry, University of California, San Francisco.

EXPRESSION OF HLA-DR BY A HUMAN MONOCYTE CELL LINE IS UNDER TRANSCRIPTIONAL CONTROL

In this attempt to determine the mechanism by which Ia induction occurs, experiments were designed to investigate the molecular events leading to expression of the human Ia molecule, HLA-DR. To accomplish this, the human monocytoid cell line U 937, which does not express any detectable HLA-DR molecules, was used. Utilizing a cDNA probe for the α chain of HLA-DR and total cellular RNA, it could be demonstrated that resting U 937 lacked detectable HLA-DR transcripts. Digestion of genomic DNA from U 937 with the isoschizomers Msp I and Hpa II followed by analysis of the restriction fragments on Southern blots demonstrated the HLA-DR α chain genes to be methylated. Addition of 5-azacytidine, an analogue of cytidine which causes hypomethylation of DNA to U 937 caused hypomethylation of HLA-DR α chain genes but did not, by itself, lead to the appearance of HLA-DR molecules on transcripts. However, treatment of U 937 with 5-azacytidine followed by addition of either culture

fluids from activated T cells or h
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Peterlin, B. M., Gonwa, T. A. a

Journal of Molecular Cell Immu

Other support: U. S. Public He

From the Howard Hughes Medic
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BOMBESIN AND VASOACTI DEVELOPING LUNG: MARK DISTRESS SYNDROME

Because there have been no
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ily increased during gestation,
concentration remained almost u
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significantly lower bombesin co
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Ghatei, M. A., Sheppard, M. N
and Bloom, S. R.

Journal of Clinical Endocrinol

Other support: The Medical Re

From the Departments of Medic
don, England.

fluids from activated T cells on human recombinant γ interferon did lead to the rapid appearance of abundant, mature HLA-DR transcripts and surface HLA-DR molecules. The results of these studies provide the first demonstration that methylation plays a role in the expression of human Ir genes and that induced expression of Ia molecules by soluble factors from T cells, including γ interferon, is accompanied by the rapid appearance of Ir gene transcripts.

Peterlin, B. M., Gonwa, T. A. and Stobo, J. D.

Journal of Molecular Cell Immunology 1:191-200; 1984.

Other support: U. S. Public Health Service.

From the Howard Hughes Medical Institute and the Department of Medicine, University of California, San Francisco.

BOMBESIN AND VASOACTIVE INTESTINAL POLYPEPTIDE IN THE DEVELOPING LUNG: MARKED CHANGES IN ACUTE RESPIRATORY DISTRESS SYNDROME.

Because there have been no immunological studies on the fetal or neonatal development of vasoactive intestinal polypeptide (VIP) in human lung or in lung diseases, in the present study the quantitative distribution of bombesin- and VIP-like immunoreactivities was determined by RIA and immunocytochemistry in regions of trachea, bronchus, and whole lung at various stages of human fetal development and in neonates, children, and adults. In addition, these two immunoreactivities were studied in infants that had died of the acute respiratory distress syndrome. Results showed that the concentration of bombesin-like immunoreactivity in the whole respiratory tract steadily increased during gestation, reaching a plateau at birth. In the lung, bombesin concentration remained almost unchanged during childhood but decreased to one tenth in the adult. In neonates with the acute respiratory distress syndrome, there was a significantly lower bombesin content in all regions of the respiratory tract compared to either normal full-term infants or 24- to 28-week-old fetuses. In related studies, immunocytochemistry localized bombesin immunoreactivity within mucosal neuroendocrine cells present in the airway epithelium throughout the respiratory tract and particularly in the intrapulmonary airways. The number of cells increased throughout gestation, reflecting the pattern found by RIA, and was greatly decreased in acute respiratory distress syndrome patients. Also, VIP concentrations were much lower than those of bombesin and did not change significantly with gestational age. In contrast to bombesin, VIP was mainly concentrated in the upper respiratory tract. In infants with the respiratory distress syndrome, the VIP content was not different from normal. These results are compatible with the possibility that bombesin-like peptides may have a role in the normal development of the human lung.

Ghatei, M. A., Sheppard, M. N., Henzen-Logman, S., Blank, M. A., *Polak, J. M.*, and Bloom, S. R.

Journal of Clinical Endocrinology and Metabolism 57(6):1226-1232; 1983.

Other support: The Medical Research Council and the Wellcome Trust.

From the Departments of Medicine and Histochemistry, Hammersmith Hospital, London, England

REGULATORY PEPTIDES: LOCALIZATION AND MEASUREMENT

The presence of five regulatory peptides, vasoactive intestinal peptide (VIP), substance P, bombesin (BN), cholecystokinin (CCK), and somatostatin, was investigated within various tissue structures, using immunocytochemistry for their localization and radioimmunoassay for the precise measurement and chemical characterization. Studies were conducted in neonatal and adult humans, rats, guinea pigs, and cats. Histologic demonstration of the diffuse neuroendocrine system in its entirety was carried out using antibodies to neuron specific enolase (NSE); delineation of the two components, neural and glial, of the autonomic nervous system was accomplished by the combined use of antisera to two brain proteins: NSE (for autonomic nerves) and S100 (for glial cells). VIP and substance P were the most abundant peptides and were localized to autonomic nerves, mainly in the upper respiratory tract, including the nasal mucosa. VIP was frequently found to be associated with secretory glands, smooth muscle and blood vessels, whereas substance P was often seen in close association with bronchial epithelium. VIP nerve fibers were seen to have a dual origin, from local cell bodies found almost exclusively in the wall of the trachea and from the sphenopalatine ganglion, where numerous VIP-containing neurons could be detected. Also, production of regulatory peptides, principally bombesin, was noted in lung endocrine tumors (i.e., small cell carcinoma) characterized immunohistologically by their high content of NSE and ultrastructurally by the presence of recognizable secretory granules. The exciting discovery of a large number of regulatory peptides in most peripheral tissues has promoted the lung to its well-deserved rank as one of the organs best provided with the diffuse neuroendocrine system. Investigations of the precise distribution and tissue localization of regulatory peptides throughout the respiratory tract and within each tissue structure provide full support of the increasingly accepted view of pulmonary regulation by messenger substances.

Polak, J. M. and Bloom, S. R.

In Becker, K. L. and Gazdar, A. F. (eds.): *The Endocrine Lung in Health and Disease*, Philadelphia, W. B. Saunders Company, 1984, pp. 300-327.

From the Departments of Medicine and Histochemistry, Hammersmith Hospital, London, England.

BIOSYNTHESIS AND ASSEMBLY OF THE α AND β SUBUNITS OF Mac-1, A MACROPHAGE GLYCOPROTEIN ASSOCIATED WITH COMPLETE RECEPTOR FUNCTION

As reported previously, Mac-1 is a macrophage surface antigen containing noncovalently associated α and β subunits of M_r = 170,000 and 95,000, respectively. To determine whether the subunits are derived from a common or separate precursor, the biosynthesis of Mac-1 was studied. [^{35}S]Methionine pulse-chase-labeled material was immunoprecipitated with either a monoclonal antibody recognizing an α chain determinant present in the associated α,β, complex or a polyclonal antiserum recognizing the α,β, complex as well as the free β subunit. In peritoneal exudate macrophages, the α subunit was derived from a precursor of M_r = 161,000 which was converted to the mature M_r = 170,000 chain with a $t^{1/2}$ of 30 to 45 min. The β subunit was derived from a M_r = 87,000 precursor which became associated with the α subunit and was converted to M_r = 95,000 with a $t^{1/2}$ of 2 h. Labeled β chain took longer than α to become

associated with the α,β, con
phage populations, correlati
phage-like tumor line, α and
β precursors were present i
ceeded processing.

Ho, M-K. and *Springer, T.*

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Other support: U. S. Public

From the Laboratory of Men
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DENDRITIC CELL AND I
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Flotte, T. J., *Springer, T.*

American Journal of Patho

Other support: National In
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From the Department of Pa
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SUREMENT

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BUNITS OF Mac-1, A
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associated with the α,β complex in a number of different types of peritoneal macrophage populations, correlating with synthesis of an excess of β. In the P388D$_1$ macrophage-like tumor line, α and β were processed with $t^1/_2$s of about 2 and 1h. Both α and β precursors were present in the complex, suggesting that complex formation preceeded processing.

Ho, M-K. and *Springer, T. A.*

The Journal of Biological Chemistry 258(5):2766-2769, 1983.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, and the Department of Pathology, Harvard Medical School, Boston.

DENDRITIC CELL AND MACROPHAGE STAINING BY MONOCLONAL ANTIBODIES IN TISSUE SECTIONS AND EPIDERMAL SHEETS

This study was undertaken to look at the tissue distribution of certain recently described murine macrophage antigens to determine whether distinctive subsets could be found and in particular, whether these antigens would also be present on some of the dendritic cells. To do this, mouse tissue sections were stained by monoclonal antibodies to macrophage antigens (Mac-1 (M1/70), Mac-2 (M3/38), Mac-3 (M3/84) with the use of immunoperoxidase. Mac-1 was located diffusely in the cytoplasm of round cells in a high percentage of alveolar macrophages, resident peritoneal and bone marrow cells, in splenic red pulp, and in rare perivascular cells in the thymus. Mac-1 was absent in epithelial cells and Langerhans cells. Mac-2 was strongly positive in many dendritic cells in the thymic medulla, more than the cortex, in paracortex and medulla of lymph nodes, sparing the follicles, and in the marginal zone of spleen. There were a few positive cells in germinal centers. Mac-2 was located in a low percentage of bone marrow and a high percentage of resident peritoneal cells. When positive in sections, Mac-3 always showed granular cytoplasmic staining. Bone marrow showed a high percentage of cytoplasmic staining (>50%), as compared with low surface staining (<1%). It was found in hematopoietic cells, and in all endothelium, including postcapillary venules and lining of sinuses. It was probable that the resulting dendritic staining pattern for Mac-3 in paracortex of lymph nodes, white and red pulp, thymic cortex, and medulla included dendritic cells other than endothelial cells. Alveolar macrophages and Kupffer cells were positive for Mac-2 and Mac-3. Mac-3 also stained bile canaliculi. Clearly different staining patterns were found in epithelial cells for Mac-2 and Mac-3 in kidney tubules, intestinal mucosal lining, bronchi, choroid plexus, and epidermis.

Flotte, T. J., *Springer, T. A.* and Thorbecke, G. J.

American Journal of Pathology III(1):112-124, 1983.

Other support: National Institutes of Health, National Institute of General Medical Sciences and the American Cancer Society Junior Faculty Fellowship.

From the Department of Pathology, New York University School of Medicine, New York, and the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston.